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Renal atrial natriuretic factor receptors in hamster cardiomyopathy

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Renal atrial natriuretic factor receptors in hamster cardiomyopathy. Hamsters with cardiomyopathy (CMO), an experimental model of congestive heart failure, display stimulated renin-angiotensin-aldosterone and enhanced sympathetic nervous activity, all factors that lead to sodium retention, volume expansion and subsequent elevation of plasma atrial natriuretic factor (ANF) by the cardiac atria. However, sodium and water retention persist in CMO, indicating hyporesponsiveness to endogenous ANF. These studies were undertaken to fully characterize renal ANF receptor subtypes in normal hamsters and to evaluate whether alterations in renal ANF receptors may contribute to renal resistance to ANF in cardiomyopathy. Transcripts of the guanylyl cyclase-A (GC-A) and guanylyl cyclase-B (GC-B) receptors were detected by quantitative polymerase chain reaction (PCR) in renal cortex, and outer and inner medullas. Compared to normal controls, the cardiomyopathic hamster's GC-A mRNA was similar in cortex but significantly increased in outer and inner medulla. Levels of GC-B mRNA were not altered by the disease. On the other hand, competitive binding studies, autoradiography, and affinity cross-linking demonstrated the absence of functional GC-B receptors in the kidney glomeruli and inner medulla. Also, C-type natriuretic peptide (CNP), the natural ligand for the GC-B receptors, failed to stimulate glomerular production of its second messenger cGMP. In CMO, sodium and water excretion were significantly reduced despite elevated plasma ANF (50.5 ± 11.1 vs. 309.4 ± 32.6 pg/ml, $P < 0.001$). Competitive binding studies of renal glomerular ANF receptors revealed no change in total receptor density, B_{max} (369.6 ± 27.4 vs. 282.8 ± 26.2 fmol/mg protein), nor in dissociation constant, K_d (647.4 ± 79.4 vs. 648.5 ± 22.9 pM). Also, ANF-C receptor density (254.3 ± 24.8 vs. 233.8 ± 23.5 fmol/mg protein), nor affinity were affected by heart failure. Inner medullary receptors were exclusively of the GC-A subtype with B_{max} (153.2 ± 26.4 vs. 134.5 ± 21.2 fmol/mg protein) and K_d (395.7 ± 148.0 vs. 285.8 ± 45.0 pM) not altered by cardiomyopathy. The increase in ANF-stimulated glomerular cGMP production was similar in normal and CMO hamsters (94- vs. 75-fold). These results demonstrate that renal ANF receptors do not contribute to the attenuated renal responses to ANF in hamster cardiomyopathy.

Atrial natriuretic peptide (ANF) is a cardiac hormone that participates in the regulation of body fluid volume through multiple mechanisms. The natriuretic and diuretic actions of ANF result from enhanced glomerular filtration rate and/or reduced tubular reabsorption of sodium and water, as well as suppression of renin, aldosterone and vasopressin [1]. The biological actions of ANF are mediated by specific guanylyl cyclase receptors, GC-A and GC-B, through generation of cGMP [2]. A third receptor, ANF-C, mediates inhibition of endothelin release and antagonism of the renin-angiotensin-aldosterone system [1] by inhibiting cAMP [3]. ANF-C receptors may also promote clearance of the peptide from the circulation [4].

In congestive heart failure (CHF), the enhanced activity of the renin-angiotensin and the sympathetic nervous systems leads to sodium retention and volume expansion, the primary stimulus for ANF release by the cardiac atria. We [5] and others [6–11] have shown that plasma ANF is elevated in CHF, and that the elevation is influenced by increased contribution of the cardiac ventricles [12] and lungs [5]. However, despite elevated plasma levels in CHF, salt and water retention persist, and the response to exogeous infusion of ANF is blunted [9, 11, 13, 14]. Mechanisms postulated for this blunted response include opposition by decreased renal perfusion pressure [15], stimulation of renin-angiotensin-aldosterone system and renal sympathetic nerve activity [16] or receptor down-regulation. Down-regulation of ANF receptors in CHF has been shown to occur in hamster lung [17, 18], human vascular beds [18] and platelets [19], but reports on renal ANF receptors are controversial. ANF receptors were decreased [20, 21], increased [22] or unchanged [11, 23] in glomeruli of animals with experimental or genetic CHF. These differences may be attributed to the various experimental designs, different models of heart failure, severity of the disease [6] or methodologies employed.

Most studies of the kidney have focused on total ANF receptors in glomeruli. However, differential modification of the proportions of ANF receptor subtypes at different segments of the nephron, may lead to important physiological effects. Furthermore, the presence of GC-B mRNA is demonstrated in rat kidney [24–26], but the sheep kidney does not exhibit functional GC-B receptors [27]. Therefore, the aims of the present studies were (i) to fully characterize renal ANF receptor subtypes at cortical and medullary sites of the normal hamster kidney, and (ii) to evaluate whether alterations in renal ANF receptors may contribute to the renal resistance to ANF in moderate to severe cardiomyopathy. Experiments were performed simultaneously on normal and cardiomyopathic hamsters (CMO), using competitive binding, autoradiography, cross-linking as well as stimulation of cGMP production. Also, the renal distribution and regulation of GC-A

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and GC-B mRNAs were assessed by reverse transcription polymerase chain reaction (RT-PCR).

Methods

Animals and tissue preparation

The cardiomyopathic hamsters (200 to 220 days old) originated from the UM-X 7.1 myopathic line, which was established by cross-breeding diseased animals from the original BIO 14.6 Golden Syrian hamster strain of the BIO-Research Institute (Cambridge, MA, USA) with unrelated healthy hamsters. These animals exhibit the same pathological changes in heart and skeletal muscles originally found in their progenitors. Their main advantage resides in the fact that the disease is rather homogeneous, with a predictable clinicopathological course starting with cardiac lesions at 30 days of age, and developing moderate to severe heart failure between 200 and 300 days [28]. At 200 days cardiomyopathic hamsters have generalized edema and pleural and abdominal effusion, altered liver size, color and firmness and an increase of heart volume. Age-matched normal Golden Syrian hamsters (Charles River, St. Constant, Quebec, Canada) served as controls. Normal and CMO hamsters were kept in individual metabolic cages at controlled room temperature under a 12 hours dark:light cycle and free access to Purina laboratory food and tap water. Daily water intake, urine excretion and body wt levels were measured on two consecutive days, and urine samples were kept for the determination of urinary sodium, potassium, ANF and cGMP. The hamsters were sacrificed by decapitation, between 8:00 and 10:00 a.m. to exclude diurnal variations. Blood (1 ml) was collected for plasma ANF measurement in prechilled tubes containing protease inhibitors to a final concentration, EDTA 10^{-3} M, phenyl methyl sulfonyl fluoride (PMSF) 10^{-5} M and pepstatin-A 0.5×10^{-5} M.

The kidneys were rapidly excised and, for the autoradiographic studies, were snap frozen in 2-methylbutane cooled by dry ice and stored at −80°C. For the preparation of membranes, the kidneys were placed in ice cold saline, decapsulated and dissected longitudinally. The inner medulla was separated, frozen in liquid nitrogen and stored at −80°C. The renal cortex was immediately processed and glomeruli were isolated by graded sieving. For membrane competitive binding and cross-linking studies, the cortical tissue was passed through a 0.5 mm grid. The paste was diluted with 0.9% saline and gently filtered by successive passage through a 200, 150, 100 and 75 μm nylon sieves. These steps were repeated until a 90 to 95% pure preparation was obtained as assessed by light microscopy. The glomeruli retained above the 75 μm sieve were washed and kept at −80°C in 50 mM Tris-HCl buffer at pH 7.4. For stimulation of cGMP production, glomeruli were similarly isolated from the renal cortex, except that saline was substituted by freshly prepared oxygenated cold Kreb's buffer at pH 7.4.

ANF was measured by radioimmunoassay (RIA) of vycor extracted plasma and Sep-Pak extracted urine as described by Gutkowska [29]. Urinary cGMP was measured directly by RIA [30]. Sodium and potassium excretions were measured by flame photometry.

Preparation of glomerular and inner medullary membranes

Glomerular membranes were prepared by homogenization of the glomeruli for one minute with a polytron followed by centrifugation at 30,000 × g at 4°C for 20 minutes. Membranes were resuspended in 50 mM Tris-HCl buffer, pH 7.4, for immediate use in competition binding radioreceptor assays.

Renal inner medullary membranes were prepared by homogenization and differential centrifugation of the tissue in a freshly prepared 50 mM Tris-HCl buffer, pH 7.4, containing 3 mM $MgCl_2$, 25 mM sucrose, 1 mM EDTA and 5 mM PMSF. The homogenates were centrifuged twice at 1500 × g at 4°C for 20 minutes. The supernatants were combined and centrifuged two times at 30,000 × g at 4°C for 45 minutes. The pellet was resuspended in 50 mM Tris-HCl buffer, pH 7.4, aliquoted and stored at −80°C.

Protein concentrations of glomerular and medullary membranes were determined spectrophotometrically by a modification of Bradford, using BSA as standard.

Competitive binding assays

Optimal binding conditions (protein and time dependence) were established in the membrane preparations, using freshly prepared monoiodinated ^{125}I-rANF and ^{125}I-tyr°CNP, at room temperature. Rat $ANF_{(99-126)}$ (rANF) and tyr°CNP (Penninsula Laboratories, Belmont, CA, USA) were iodinated by the lactoperoxidase method and purified by HPLC as previously described [29].

Competition binding studies were performed simultaneously on renal membranes obtained from normal and cardiomyopathic hamsters. The assay buffer consisted of 50 mM Tris-HCl buffer, pH 7.4, containing 0.1% bacitracin, 0.5 mM PMSF, 5 mM $MgCl_2$, 25 mM $MnCl_2$, 1 mM EDTA and 0.4% BSA. Aliquots of membrane preparations were incubated at 22°C, with 20,000 cpm of ^{125}I-rANF or ^{125}I-tyr°CNP, without and with increasing concentrations (10^{-12} to 10^{-6} M) of specific displacing peptides, $rANF_{(99-126)}$, c-type natriuretic peptide (CNP-22) and synthetic $C\text{-}ANF_{(102-121)}$ (Peninsula Laboratories) in a total volume of 0.2 ml. The reaction was stopped by adding 3 ml of cold 50 mM Tris-HCl buffer, pH 7.4, and rapid filtration of the membrane bound tracer on Whatman GF/C filters (Millipore, Mississauga, Ontario, Canada) presoaked in 1% polyethylenimine (PEI). The filters were rinsed twice with 3 ml of 50 mM Tris-HCl buffer, pH 7.4, dried and counted in a Hewlett-Packard gamma counter. Binding in the presence of 10^{-6} M of the displacing peptides rANF, CNP and $C\text{-}ANF_{(102-121)}$ was considered nonspecific.

Affinity cross-linking studies

Glomerular and inner medullary membranes (250 μg of protein) were incubated with ^{125}I-rANF (10^6 cpm) in binding buffer containing 50 mM in Tris-HCl buffer at pH 7.2, 0.2 mM PMSF, 20 μg/ml leupeptin, 20 μg/ml aprotinin, 10 mM $MgCl_2$ and 30 mM NaCl. Specificity of binding was determined by the addition of 10^{-7} M rANF, CNP and $C\text{-}ANF_{(102-121)}$ to the reaction mixture. After 90 minutes of incubation at 4°C, and centrifugation at 30,000 × g for 15 minutes, the pellet was resuspended in 250 μl of the binding buffer and 250 μl of distilled water. The receptors were cross-linked by incubation for 30 minutes at 4°C with 25 μl/tube disuccinyl suberate (3.68 mg/ml in dimethyl sulfoxide). Then the reaction was stopped by addition of 25 μl ammonium acetate and centrifugation at 30,000 × g for 15 minutes at 4°C. The pellet was resuspended in sample buffer containing 2% β-mercaptoethanol, boiled for 10 minutes (denatured under reducing conditions) and separated by polyacrylamide gel electrophoresis. High molecular weight standards ranging from 45 to 200

kD (Biorad, Richmond, CA, USA) were used. The gel was stained with Coomassie blue R-250, dried, exposed to a phosphor-sensitive screen for three days then scanned with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA, USA).

Stimulation of cGMP

The production of cGMP was stimulated by rANF and CNP, in freshly isolated renal glomeruli of normal and CMO hamsters simultaneously. Glomerular aliquots (100 μg) were preincubated in a 95% O_2 and 5% CO_2 atmosphere, at 37°C for 15 minutes, then for two minutes in 10 mM isobutylmethylxanthene (IBMX). Rat ANF and CNP were added to the aliquots in a final concentration of 5×10^{-8} M, and incubated for 90 seconds. The reaction was stopped by the addition of 2 ml of 1 N perchloric acid and cooling in liquid N_2. On the assay day, the samples were sonicated for 15 seconds then centrifuged at 12,000 × g at 4°C. cGMP was determined in the supernatants by radioimmunoassay as previously described by Tremblay [30].

Autoradiography

Cryostat whole kidney sections (20 μm) from six normal and six CMO hamsters, were cut and mounted on acid-washed gelatinized slides, then placed overnight in a partial vacuum at −4°C. Slides were stored in boxes with Drierite at −80°C until the autoradiographic procedures were performed [31]. Optimal binding conditions (amount of radiolabeled ligand and incubation time) were determined in preliminary studies. Sections from normal and CMO kidneys were assayed simultaneously. Duplicate slides were brought to room temperature in incubation buffer containing 50 mM Tris-HCl, pH 7.4, and 0.1% PEI for 15 minutes. The effect of prior occupancy was eliminated by placing the slides in acidic buffer (40 mM sodium acetate, 150 mM NaCl, pH 5.0) for 10 minutes followed by washing with Tris-HCl buffer at pH 7.4. Then, the slides were incubated with 50 pM ^{125}I-rANF for one hour at room temperature. The binding buffer consisted of 50 mM Tris-HCl buffer, pH 7.4, 150 mM NaCl, 40 mg/ml bacitracin, 0.5% BSA, 0.2 mM PMSF and 20 μg/ml leupeptin. The sections were washed four times, two minutes each, with ice-cold Tris-HCl buffer, pH 7.4, at 4°C. The slides were finally rinsed with distilled water to remove the salts and dried rapidly under a stream of cold air.

Nonspecific binding was determined on adjacent sections under identical incubation conditions except for the addition of 10^{-6} M and 10^{-7} M rANF. GC-B and ANF-C receptors were identified by addition of 10^{-7} M CNP and C-ANF$_{(102-121)}$, respectively. Localization of the binding sites of CNP was also determined in sections similarly incubated and displaced with CNP as above, but after saturation of ANF-C receptors with 10^{-7} M C-ANF$_{(102-121)}$.

For direct evaluation of the GC-B receptors, another set of slides were treated as above, except that the sections were incubated with ^{125}I-tyr°CNP (100 pM) without and with displacing peptides, rANF and C-ANF$_{(102-121)}$ at 10^{-7} M, CNP at 10^{-6} M and 10^{-7} M as well as by 10^{-7} M CNP after saturation of the ANF-C receptors with 10^{-7} M C-ANF$_{(102-121)}$.

The dried tissue sections were exposed in phosphor-sensitive cassette for 48 hours, then scanned, visualized and quantified by PhosphorImager (Molecular Dynamics).

RNA extraction

Kidneys of three normal and three CMO hamsters were dissected and the inner medulla, outer medulla and cortex were separated then placed in liquid N_2 and stored at −80°C. Total RNA was extracted by the acid guanidinum-thiocyanate-phenol-chlorophorm method [32]. Additionally, RNA was treated with DNAse I. The integrity of the preparations was verified by gel electrophoresis and RNA concentrations were measured by UV spectrophotometry.

First strand cDNA synthesis and quantification reaction product

Total RNA (2 μg) was annealed with 0.5 μg of random hexamer and reverse transcribed in 50 mM Tris-HCl buffer (pH 8.3), containing 7 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.5 mM dNTP, 40 units RNAsin (Pharmacia Biotech, Uppsala, Sweden), 200 units Moloney murine leukemia virus reverse transcriptase (Gibco BRL, Burlington, Ontario, Canada), in total volume of 40 μl. The reaction was incubated for one hour at 37°C. To reduce variation in RNA input, length and quantification of the product, newly synthesized cDNA was labeled by the addition of 1 μCi/reaction of 2500 Ci/mM ^{32}P-dCTP (Amersham, Oakville, Ontario, Canada) to mastermix. A 3 μl aliquot of reverse-transcription reaction was denatured at 95°C for five minutes and then electrophoresed on 1% agarose gel. The gel was dried on autoradiographic film and counts over 400 bp in length were integrated for each sample using Image-Quant analysis software (Molecular Dynamics). Input of cDNA samples in each PCR reaction was normalized according to the measurements of radioactive reverse-transcription reaction product [33].

Polymerase chain reaction (PCR) amplification of GC-A and GC-B transcripts

cDNA preparations were mixed with fixed amount of mutated DNA, containing mismatched bases, which create an EcoRI site [24, 34]. PCR amplification was performed with the following primers:

GC-A forward, 5′-AGTGTTACCATCTACTTCAGTGATAT-TGTG-3′;

GC-A reverse, 5′-CTCGAAACCATCGAACTCTTCCAGCA-CACAGC-3′;

GC-B forward, 5′-GGTACCAGCATATTGGACAACCTC-3′;

GC-B reverse, 5′-CAGGAGTCCAGGAGGTCCTTTTCG-3′.

A volume of 50 μl of 50 mM Tris-HCl buffer (pH 8.4) containing 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 40 pmol of each primer, 2.5 units of *Taq* DNA polymerase (Gibco BRL) and 10 μCi of ^{32}P-dCTP was incubated for 30 cycles of one minute of denaturation at 94°C, one minute annealing at 65°C, and two minutes polymerization at 72°C. Ten microliters of each sample were digested at 37°C for one hour with 10 units of *Eco*RI. The digested samples were electrophoresed on 1.5% agarose gel that gave upper non-digested bands derived from endogenous cDNA (GC-A 474 bp, and GC-B 762 bp), and lower digested bands derived from mutated cDNA (GC-A 283 bp and 191 bp; GC-B 385 and 377 bp, respectively).

Table 1. Biochemical and hormonal parameters in normal and cardiomyopathic (CMO) hamsters

	Normal	CMO
Body weight *g*	163.2 ± 1.9	137.3 ± 3.3[c]
Water intake *ml/day*	23.4 ± 4.8	10.4 ± 1.1[c]
Water intake *ml/day/% body wt*	14.3 ± 2.9	7.5 ± 0.6[a]
UV *ml/day*	9.1 ± 3.0	2.0 ± 0.4[c]
UV *ml/day/% body wt*	6.2 ± 1.8	1.4 ± 0.3[a]
$U_{Na}V$ *mmol/day*	0.68 ± 0.11	0.33 ± 0.05[b]
$U_{Na}V$ *mmol/day/% body wt*	0.42 ± 0.07	0.24 ± 0.03[b]
U_KV *mmol/day*	1.59 ± 0.19	0.94 ± 0.17[b]
U_KV *mmol/day/% body wt*	0.97 ± 0.11	0.68 ± 0.11[a]
U_{cGMP} *mmol/day*	14.3 ± 1.1	18.5 ± 2.3
U_{cGMP} *mmol/day/% body wt*	9.2 ± 0.5	13.5 ± 1.7[b]
U_{ANF} *pg/day*	2308 ± 280	1462 ± 275[a]
U_{ANF} *pg/day/% body wt*	1414 ± 169	1064 ± 196
Plasma ANF *pg/ml*	50.5 ± 11.1	309.4 ± 32.6[c]

Abbreviations are: % body wt, percent body weight; UV, urinary volume; $U_{Na}V$, urinary sodium excretion, U_KV, urinary potassium excretion; U_{cGMP}, urinary cGMP excretion, U_{ANF}, urinary ANF excretion.
[a] $P < 0.05$ vs. normal, [b] $P < 0.01$ vs. normal, [c] $P < 0.001$ vs. normal

Data analysis

The equilibrium dissociation constant (K_d) and maximum binding capacity (B_{max}) for the ligands used in the competitive binding radioreceptor studies were calculated using the LIGAND computer program (Elsevier-Biosoft, Cambridge, UK). Data storage, graphical output and statistical analyses were accomplished using RS1 data analysis software (BBN, Cambridge, MA, USA). All data were assessed using analysis of variance followed by Bonferroni's test for repeated measurements. Statistical significance was taken as $P < 0.05$. All data are reported as mean ± SEM.

Results

Basal biochemical and hormonal parameters determined in 12 normal and 12 CMO hamsters are shown in Table 1. Compared to their normal age-matched controls, the hamsters with moderate to severe cardiomyopathy exhibited a reduced body weight, water intake, urinary volume and excretion of sodium and potassium. The reduction in volume and electrolyte excretion persisted after normalizing the values to individual body weights. These changes were accompanied by marked elevation in plasma ANF but reduced urinary ANF (2308 ± 280 vs. 1462 ± 275 pg/ml, $P < 0.05$). Urinary cGMP tended to increase in CMO, but reached significance after normalizing the levels to individual body weight (9.2 ± 0.5 vs. 13.5 ± 1.7 nmol/day/% body wt, $P < 0.01$).

Competitive binding assays

Glomerular ANF receptors. Competitive binding assays were performed by incubating 50 μg of glomerular membrane protein with ^{125}I-rANF for two hours at room temperature. Figure 1 illustrates curves plotted from data of binding assays of glomerular membranes of CMO ($N = 7$ groups of 10 hamsters/group) and corresponding normal hamsters ($N = 7$ groups), represented as $\%B/B_o$, where B and B_o represent, respectively, binding with and without displacing peptides. Bound ^{125}I-rANF was progressively inhibited by increasing concentrations of rANF and to a lesser extent by C-$ANF_{(102-121)}$ and CNP. These results indicate that the glomerular receptors are of the guanylyl cyclase and the clearance subtypes. The presence of GC-B receptor subtype was questionable because the similarity of inhibition by CNP and C-$ANF_{(102-121)}$ raised the possibility that CNP binding could have been mediated by the ANF-C clearance receptors.

Kinetic parameters obtained from the displacement curves revealed that, compared to normal controls, the cardiomyopathic hamsters exhibited similar total glomerular ANF binding sites, B_{max} (369.6 ± 27.4 vs. 282.8 ± 26.2 fmol/mg protein), and dissociation constant, K_d (647.4 ± 79.4 vs. 648.5 ± 22.9 pM). Also, ANF-C receptor density (254.3 ± 24.8 vs. 233.8 ± 23.5 fmol/mg protein) and affinity were not affected by heart failure.

Inner medullary ANF receptors. Figure 2 illustrates curves obtained from competitive binding receptor assays of renal inner medullary membranes (70 μg) of six groups each (10 hamsters/group) of normal and CMO hamsters. Increasing concentrations of unlabeled rANF inhibited progressively binding of ^{125}I-rANF to inner medullary membranes. But CNP and C-$ANF_{(102-121)}$ failed to inhibit binding and represented less than 10% at 10^{-6} M concentrations. These results indicated that hamster inner medullary membranes are exclusively of the GC-A receptor subtype. Kinetic parameters obtained from the competitive binding curves showed that B_{max} (153.2 ± 26.4 vs. 134.5 ± 21.2 fmol/mg protein) and K_d (395.7 ± 148.0 vs. 285.8 ± 45.0 pM) were not altered by cardiomyopathy. On the other hand, under identical conditions ^{125}I-tyr°CNP failed to bind to renal inner medullary membranes (data not shown), confirming the absence of GC-B receptors.

Autoradiographic studies

The results obtained from glomerular and inner medullary membranes binding studies were further confirmed by autoradiography. Figure 3 shows slices of whole kidneys obtained from normal and CMO hamsters, where the blue to red colored scale represents increasing binding. In the normal hamster, high ^{125}I-rANF binding was shown in all the kidney structures, but was mostly pronounced in cortical and inner medullary regions. Quantification by PhosphorImager revealed that nonspecific binding in the cortex, determined in the presence of 10^{-6} M rANF was less than 17% in both normal and CMO hamsters. In normal hamsters, cortical binding was displaced by 10^{-7} M rANF, C-$ANF_{(102-121)}$ and CNP and represented 77%, 48% and 55% of total binding, respectively. Similar findings were obtained in the CMO where cortical displacement with these peptides represented 85%, 48% and 49% of corresponding total cortical binding. Not shown in the Figure is that after saturation of the ANF-C receptors with 10^{-7} M C-$ANF_{(102-121)}$, CNP did not result in further displacement. The equal displacement obtained with CNP and C-$ANF_{(102-121)}$, and the lack of additive effect of CNP and C-$ANF_{(102-121)}$ suggest that both ligands were binding to the same receptor, namely ANF-C.

High binding of ^{125}I-rANF was observed at the inner medulla and at the tip of the papilla (Fig. 3). Specific binding represented 80% of total inner medullary binding in normal and CMO hamsters. But C-$ANF_{(102-121)}$ and CNP failed to displace bound ^{125}I-rANF from inner medullary structures, consistent with the absence of ANF-C and GC-B receptors (Fig. 3).

These results were further confirmed by using ^{125}I-tyr°CNP as the labeled ligand (Fig. 4). High specific binding of ^{125}I-tyr°CNP was observed in the cortex, while binding was completely absent from inner medulla. (Fig. 4). Cortical binding was equally displaced by 10^{-7} M CNP, rANF and C-$ANF_{(102-121)}$ implying that ^{125}I-tyr°CNP was binding to the ANF-C receptor.

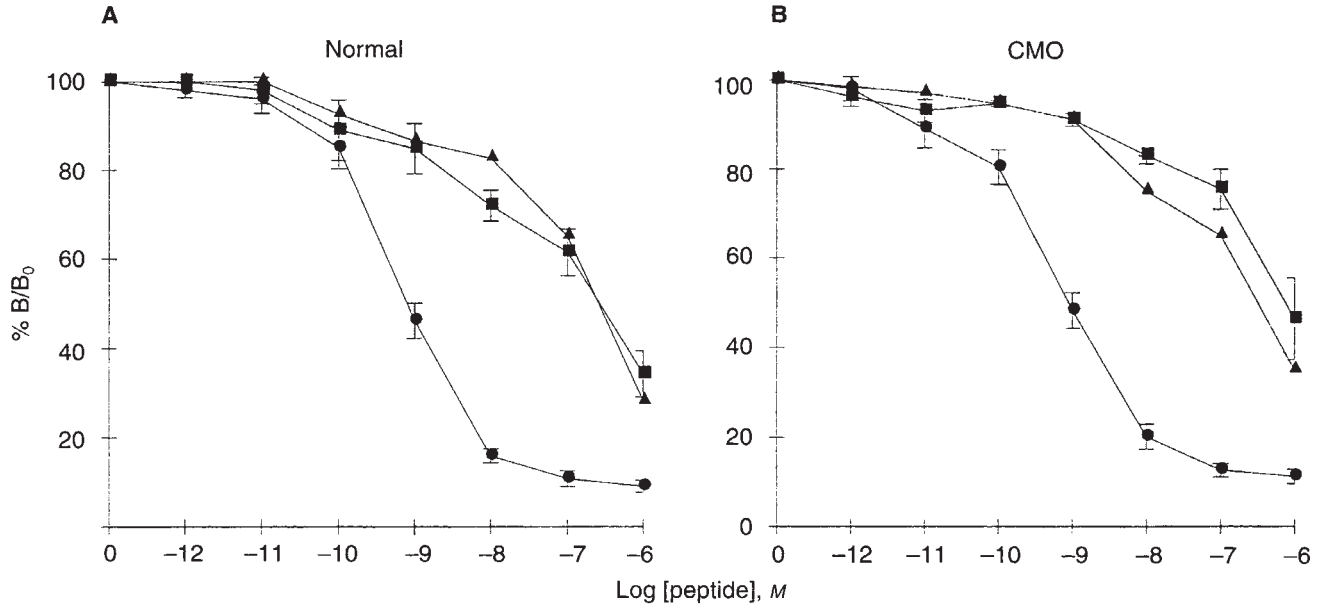

Fig. 1. *Competitive binding curves obtained by incubating renal glomerular membranes with ^{125}I-rANF for two hours at 22°C in the presence of increasing concentrations (10^{-12} to 10^{-6} M) of rANF (●) and C-ANF$_{(102-121)}$ (■) and CNP (▲) in normal and cardiomyopathic (CMO) hamsters.* Curves are plotted as percent B/B_0 where B and B_0 represent specific binding in the presence or absence of displacing peptides. Each point represents mean ± SEM of 7 determinations in duplicate.

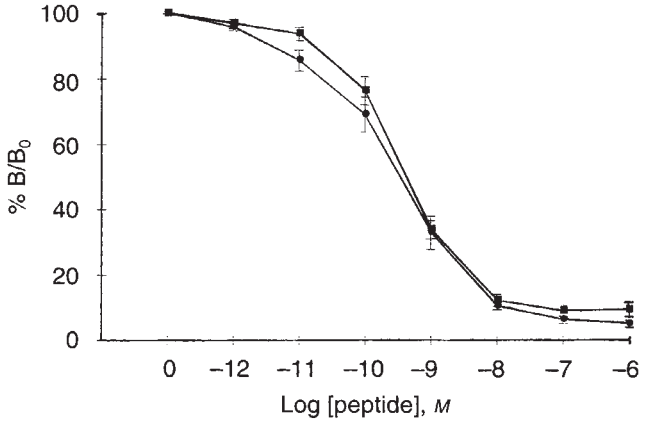

Fig. 2. *Competitive binding curves obtained by incubating renal inner medullary membranes with ^{125}I-rANF for two hours at 22°C in the presence of increasing concentrations of unlabeled rANF in normal (●) and cardiomyopathic (■) hamsters.* Curves are plotted as percent B/B_0 where B and B_0 represent specific binding in the presence or absence of displacing peptides. Each point represents mean ± SEM of 6 determinations in duplicate.

Affinity cross-linking

The identity of glomerular ANF receptors was verified by performing affinity cross-linking of renal glomerular membranes (Fig. 5) of normal (lanes 1 to 4) and CMO (lanes 5 to 8) hamsters. Figure 5 shows the presence of two bands corresponding to molecular weights 130 and 66 kD (lanes 1 and 5). The high molecular weight bands were inhibited by 10^{-7} M rANF (lanes 2 and 6), but not by 10^{-7} M CNP (lanes 3 and 7), nor 10^{-7} M C-ANF$_{(102-121)}$ (lane 4 and 8). On the other hand, the low molecular weight bands were inhibited by rANF (lanes 2 and 6), and C-ANF$_{(102-121)}$ (lane 4 and 8), but not by CNP (lanes 3 and 7). These results indicate that glomeruli exhibit GC-A and ANF-C but not GC-B receptors. The third band below the 66 kD was not displaced by the peptides and may be nonspecific.

Cross-linking of inner medullary membranes (Fig. 6) of normal (lanes 1 to 4) and CMO hamsters (lanes 5 to 8), revealed the presence of one band corresponding to the high molecular weight ANF receptor (130 kD). The band was displaced by rANF (lanes 2 and 6) but not by C-ANF$_{(102-121)}$ (lanes 3 and 7) nor CNP (lanes 4 and 8) at 10^{-7} M concentrations.

In vitro stimulation of cGMP production

Baseline cGMP production from isolated glomeruli obtained from 3 groups of normal and CMO animals, tended to be less in CMO (0.27 ± 0.09 vs. 0.17 ± 0.04 pmol/mg protein). Upon stimulation with 5×10^{-8} M rANF, cGMP production was significantly less in CMO (16.57 ± 1.31 vs. 11.37 ± 0.83 pmol/mg protein, $P = 0.02$). However, when the data were presented as percent increase from basal levels, the increase in normal hamsters was not significantly diffrent from that in CMO (94- vs. 75-fold). On the other hand, CNP at 5×10^{-8} M concentration failed to stimulate cGMP production in glomeruli of normal (0.26 ± 0.09 pmol/mg protein) and CMO hamsters (0.11 ± 0.02 pmol/mg protein) confirming the absence of functional GC-B receptors.

mRNA of GC-A and GC-B receptors

The presence of GC-A and GC-B mRNA was detected by RT-PCR throughout the kidney regions, cortex, outer and inner medulla. Figure 7 shows representative PhosporImager density bands of PCR products after *Eco*RI digestion and gel electrophoresis. Among the segments, inner medulla showed highest

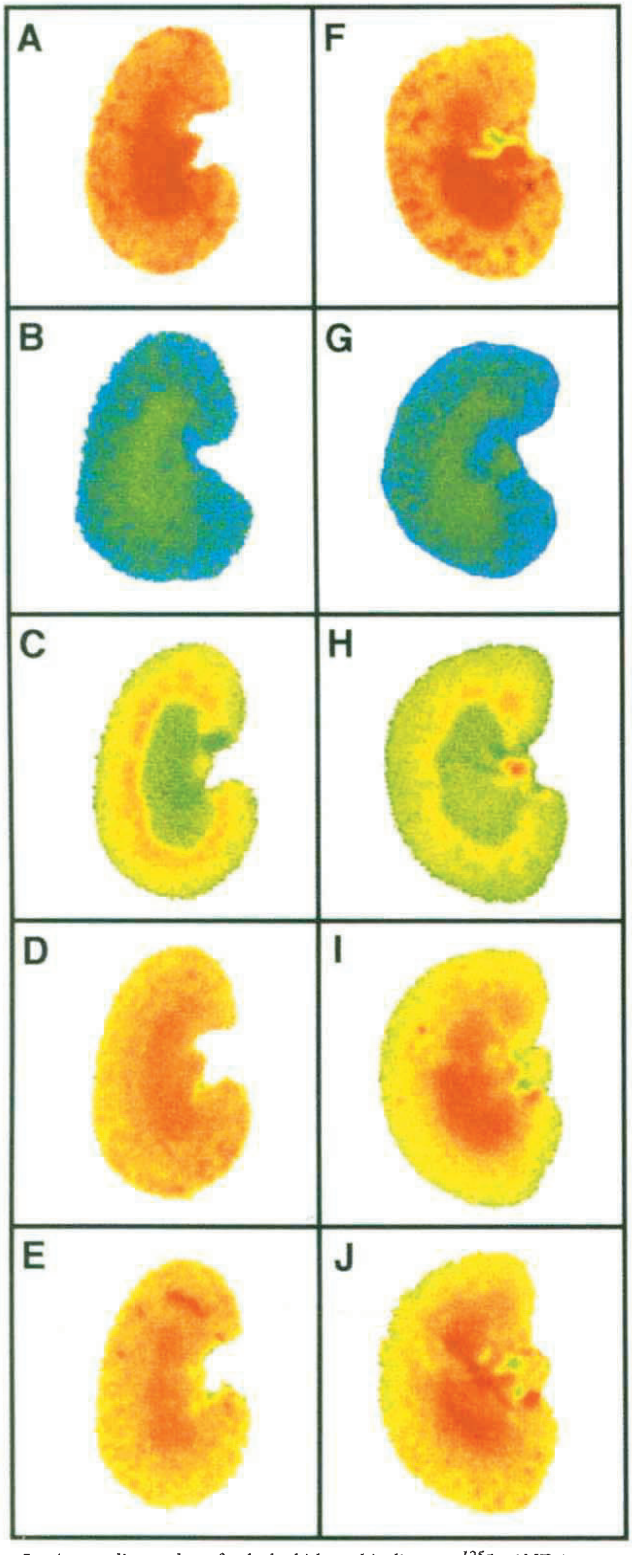

Fig. 3. *Autoradiography of whole kidney binding to ^{125}I-rANF in normal (**A-E**) and cardiomyopathic (**F-J**) hamsters.* Total binding (A and F), inhibition with 10^{-6} M rANF (B and G), 10^{-7} M rANF (C and H), 10^{-7} M CNP (D and I) and 10^{-7} M C-ANF$_{(102-121)}$ (E and J). Reproduction of this figure in color is made possible by a grant from Pfizer Canada, Inc., Quebec, Canada.

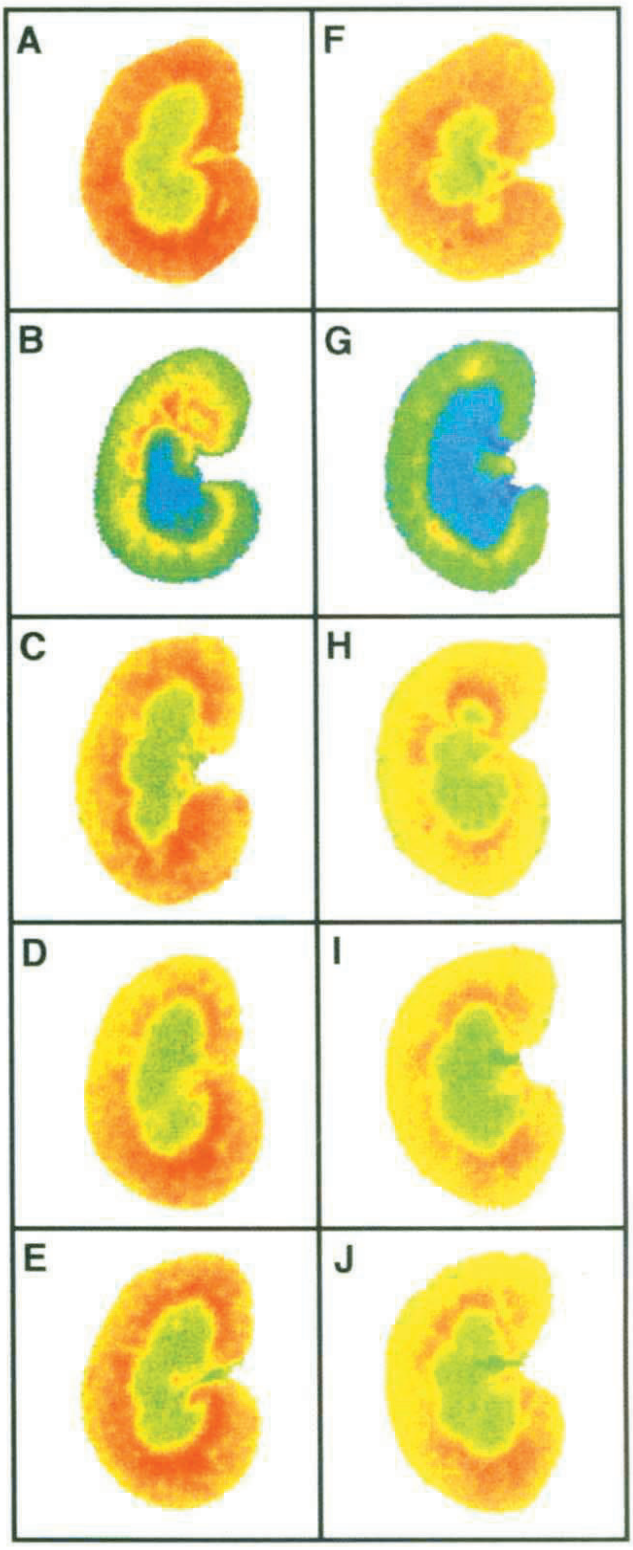

Fig. 4. *Autoradiography of whole kidney binding to ^{125}I-tyr°CNP in normal (**A-E**) and cardiomyopathic (**F-J**) hamsters.* Total binding (A and F), inhibition with CNP at 10^{-6} M (B and G) and 10^{-7} M (C and H), 10^{-7} M C-ANF$_{(102-121)}$ (D and I), 10^{-7} M rANF (E and J). Reproduction of this figure in color is made possible by a grant from Pfizer Canada, Inc., Quebec, Canada.

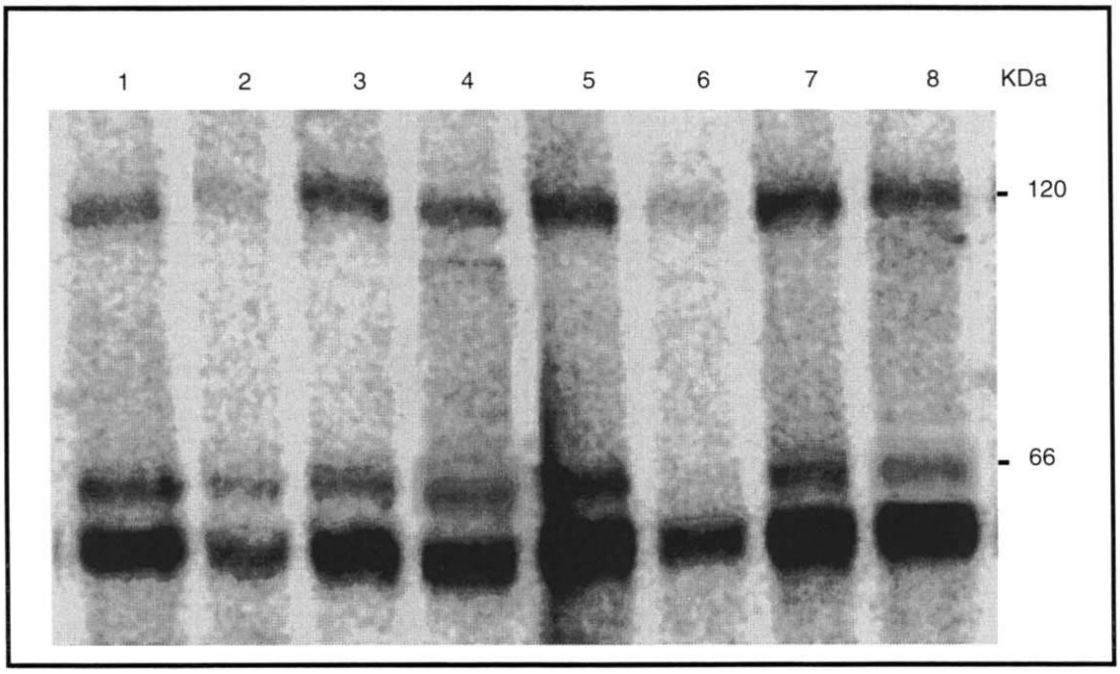

Fig. 5. *Affinity cross-linking of ^{125}I-rANF to renal glomerular membranes of normal (lanes 1 to 4) and cardiomyopathic (lanes 5 to 8) hamsters in the absence (lanes 1 and 5) and presence of 10^{-7} M rANF (lanes 2 and 6), CNP (lanes 3 and 7) and C-ANF$_{(102-121)}$ (lanes 4 and 8).* Two bands corresponding to 120 kD and 66 kD molecular mass were observed.

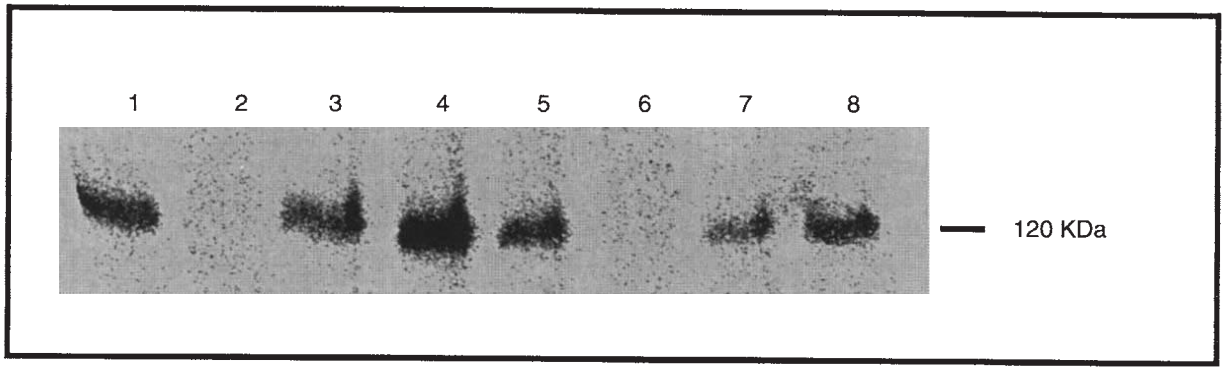

Fig. 6. *Affinity cross-linking of ^{125}I-rANF to renal inner medullary membranes of normal (lanes 1 to 4) and cardiomyopathic hamsters (lanes 5 to 8) in the absence (lanes 1 and 5) and presence of 10^{-7} M rANF (lanes 2 and 6), CNP (lanes 3 and 7) and C-ANF$_{(102-121)}$ (lanes 4 and 8).* A single band corresponding to the high molecular weight standard (120 kD) was observed.

signal of GC-A mRNA, while GC-B mRNA was equally expressed in the various segments. Quantification of the bands revealed that, compared to normal controls, the cardiomyopathic hamster GC-A mRNA was similar in cortex but significantly increased in outer and inner medulla. However, the levels of GC-B transcripts were not altered by the disease.

Discussion

The present studies show that despite the expression of GC-B receptors, the hamster kidney lacks functional GC-B receptors. Renal glomeruli exhibit GC-A and ANF-C, while the inner medullary receptors are exclusively of the GC-A subtype. These findings are shown by competitive binding, affinity cross-linking, autoradiography and by *in vitro* stimulation of cGMP production. In CMO, the level of GC-A transcript is enhanced in outer and inner medullary regions, but kinetic parameters (density and affinity) and biological activity (cGMP production) of renal ANF receptors are not altered.

Three ANF receptors have been identified by molecular cloning and sequence analysis [1, 35]. Two subtypes, GC-A and GC-B have a large extracellular binding domain and an intracellular guanylyl cyclase domain within a single polypeptide chain of 120 to 140 kD. Distinction between these receptors is based on their ligand selectivity. GC-A shows higher affinity to ANF, while GC-B binds CNP preferentially [1, 35]. The third, ANF-C receptor which is most widely distributed of the three ANF receptors, consists of a large extracellular domain and a short cytoplasmic tail that lacks the cyclase activity. ANF-C forms a homodimer that can be reduced to ~66 kD molecular mass with β-mercaptoethanol. ANF-C receptor shows less structural requirements, as it

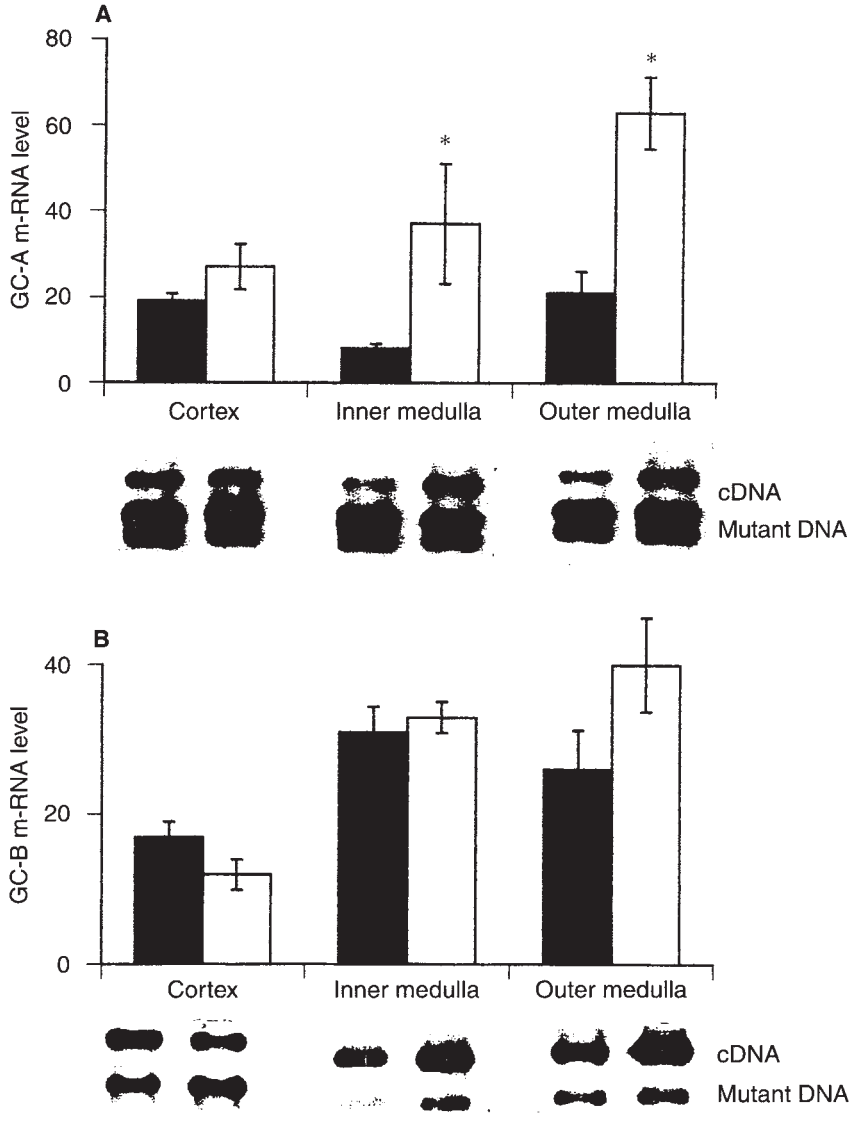

Fig. 7. *Representative PhosphorImager density bands of PCR products after EcoRI digestion and gel electrophoresis of GC-A and GC-B mRNA detected in renal cortex, outer and inner medulla of normal and cardiomyopathic hamsters.* The upper non-digested bands represent endogenous cDNA (GC-A, 474 bp; GC-B, 762 bp). Lower digested bands represent mutated cDNA (GC-A, 283 and 191 bp; GC-B, 385 and 377 bp, respectively). Results are presented as bar graphs and expressed as percentage of the sum of two bands taken as 100% for each amplified sample from 3 determinations. Symbols are: (■) normal; (□) CMO.

binds to all the natriuretic peptides and ANF metabolites, but shows higher affinity to C-ANF$_{(102-121)}$, a synthetic 5 amino acid deleted ring ANF analog [4, 35].

Our present findings confirm that the kidney cortex and papilla are rich in ANF binding sites [4] and that the cortex possesses both guanylyl cyclase receptors and clearance receptors, while the inner medulla possesses only guanylyl cyclase ANF receptors [1, 27, 35]. In addition, the present detection of GC-A and GC-B transcripts in all regions of the hamster kidney is consistent with the widespread distribution of GC-A and GC-B mRNA detected by PCR throughout the rat nephron [24, 25, 36]. However, using competitive binding receptor assays, autoradiography, cross-linking as well as *in vitro* stimulation of cGMP production, we demonstrate the absence of functional GC-B receptors from hamster glomeruli and inner medulla. This finding does not necessarily contradict our present finding and earlier reports of the presence of GC-B transcripts in the kidney. The level of expression does not always correlate with the number of functional cell surface receptors [37], since mRNA level is not the only determinant of the amount and function of the protein. Other factors involved include translational efficiency, protein processing and turnover, transport and insertion into the plasma membrane [34]. On the other hand, the lack of correlation may be also attributed to the sensitivity of the techniques used. Yamamoto et al [38] were able to detect GC-B mRNA along the bovine kidney nephron using RT-PCR, but not by *in situ* hybridization. Consequently, it is possible that the binding assays, autoradiography, cross-linking and stimulation of cGMP production, may have failed to detect very low amounts of the receptor whose transcript was detected by PCR amplification. However, consistent with the present results, the dissociation between GC-B mRNA levels and activity of GC-B in stimulating cGMP production was also observed in vascular smooth muscle cells [34], and in uterine tissue [39]. Furthermore, whereas the presence of functional renal

GC-B receptors in rats is controversial [36, 40, 41], the lack of measurable amounts of GC-B receptors was shown in renal glomeruli of sheep [27] and recently in hamsters [41].

Similar to previous observations in humans [8, 9] and experimental animals [10, 11, 23], the cardiomyopathic hamsters in the present study showed reduced diuresis and natriuresis and elevated plasma ANF, suggesting an inability to excrete excess sodium and a decrease in renal responsiveness to endogenous ANF. The renal resistance to ANF in cardiomyopathy cannot be explained by reduced activity of the peptide. Since urinary cGMP, the biological marker of ANF activity [42], was significantly elevated, a finding confirmed by others [10]. The dissociation between ANF-induced urinary cGMP and sodium excretion also observed in young rats [43], could reflect an overactive but inadequate ANF system, probably due to altered hemodynamics and neurohumoral reflexes in CHF.

The mechanisms underlying the hyporesponsiveness to ANF may also include receptor down regulation. Elevated plasma angiotensin II and enhanced renal nerve activity in CMO should result in down-regulation of ANF receptors [44]. Contrary to our expectations, competitive binding, affinity cross-linking and autoradiographic studies showed that ANF receptors at glomerular and inner medullary sites are not changed in cardiomyopathic hamsters. These results are in agreement with Bianchi et al [7] and with Isnard et al [11] who reported that glomerular ANF receptors are not altered by moderate to severe cardiomyopathy in the hamster [7], and experimental CHF in the rabbit [11]. In contrast, Cachofeiro et al [22] reported up-regulation in glomerular ANF receptors in cardiomyopathic hamsters. The increased receptor density reported in the latter study was accompanied by reduced affinity, which should result in no net change in ANF receptor binding capacity, and therefore would be similar to the present results. This observation was later confirmed by the same group [23] in rats with CHF caused by A-V shunt. On the other hand, our present findings do not confirm previous reports showing decreased inner medullary ANF receptors in rats with chronic heart failure [21, 45].

Glomeruli of CMO hamsters showed reduced *in vitro* production of cGMP, at baseline and after stimulation with ANF, which may suggest an absolute down-regulation of the glomerular receptors occurring at the second messenger level. But the reduction disappeared when cGMP production was normalized to basal levels, suggesting an intact receptor sensitivity [11]. The possibility of a mechanism distal to the production of cGMP appears to be very likely. Valentin et al [46] demonstrated that the blunted natriuretic response to volume expansion and hence elevated endogeous ANF in experimental nephrotic syndrome in rats, resulted from enhanced hydrolysis of cGMP by the enhanced phosphodiesterase activity. This effect was reversed by phosphodiesterase inhibitors [46].

Urinary ANF was reduced in CMO hamsters and correlated with the diminished sodium excretion, but not with elevated plasma ANF nor with the enhanced urinary cGMP excretion. We are presently unable to explain this result. However, reduced urinary ANF may reflect reduction in urodilatin, the renal natriuretic peptide. Urodilatin shares with ANF the carboxyterminus [35] that is recognized by our RIA antibody [29], but differs by 4 amino acid extended amino terminus, that has no effect on its immuoreactivity. We have shown in an earlier study in the sheep, that plasma ANF does not appear in urine in an intact form [47], because of the unsaturable and high peptidase activity (NEP) present in the brush border of the renal proximal tubule [48]. Urodilatin, secreted at the inner medullary sites [49] binds to and activates renal ANF receptors [50], but unlike ANF, it escapes this enzymatic degradation [51]. Further studies are required to clarify the role of urodilatin in CHF but a plausible possibility is that for more efficient conservation of volume, suppression of urodilatin and other natriuretic peptides in CHF may further reduce the natriuretic responses to ANF. Reduction in renal kinins were reported to mediate renal resistance to ANF in sodium-retaining chronic caval dogs [52].

Taken together, the unchanged to over-expressed GC-A receptors as well as the intact second messenger system, may suggest that the renal ANF system is preserved in CHF. The attenuation of renal responses to ANF may be explained by reduced delivery of ANF to the distal nephron [52], the overriding effects of diminished renal perfusion pressure [15] and the antagonistic actions of angiotensin II [53, 54], as well as decreased production of intrarenal urodilatin, all factors that favor sodium reabsorption. In acute CHF in dogs, restoring renal perfusion pressure restored the renal natriuretic response to ANF [15]. Intrarenal infusion of angiotensin II attenuated the natriuretic response to ANF [53] problably by stimulating the hydrolysis of cGMP by a Ca^{2+}-activated cGMP phosphodiesterase [46, 54]. Also, infusion of urodilatin in rats, produced significant dose-dependent increases in urinary flow, glomerular filtration rate, and in absolute and fractional sodium excretions [10].

In summary, the present studies demonstrate the absence of functional GC-B receptors in glomeruli and inner medulla of the hamster kidney. Also, the renal ANF system is shown to be intact in hamster cardiomyopathy. The renal resistance to ANF may be secondary to the opposing actions of reduced pressure and enhanced angiotensin II activity and/or to an intracellular defect beyond the second messenger system. Further studies are required to clarify whether a post-receptor defect exists in cardiomyopathy.

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References

1. JAMISON RL, CANAAN-KUHL S, PRATT R: The natriuretic peptides and their receptors. *Am J Kidney Dis* 20:519–530, 1992
2. HAMET P, TREMBLAY J, PANG SC, GARCIA R, THIBAULT G, GUTKOWSKA J, CANTIN M, GENEST J: Effect of native and synthetic atrial natriuretic factor on cyclic GMP. *Biochem Biophys Res Commun* 123:515–527, 1984
3. ANAND-SRIVASTAVA M, GUTKOWSKA J, CANTIN M: The presence of atrial natriuretic factor receptors of ANF-R2 subtype in rat platelets. *Biochem J* 278:211–217, 1991

4. MAACK T, SUZUKI M, ALMEIDA FA, NUSSENZVEIG D, SCARBOROUGH RM, MCENROE GA, LEWICKI JA: Physiological role of silent receptors of atrial natriuretic factor. *Science* 238:675–678, 1987
5. GUTKOWSKA J, NEMER M, SOLE NJ, DROUIN J, SIROIS P: Lung is an important source of atrial natriuretic factor in experimental cardiomyopathy. *J Clin Invest* 83:1500–1504, 1989
6. DING J, THIBAULT G, GUTKOWSKA J, GARCIA R, KARABATSOS T, JASMIN G, GENEST J, CANTIN M: Cardiac and plasma atrial natriuretic factor (ANF) in experimental congestive heart failure. *Endocrinology* 121:248–257, 1987
7. BIANCHI C, THIBAULT G, WROBEL-KONRAD E, DE LEAN A, GENEST J, CANTIN M: Atrial natriuretic factor binding sites in experimental congestive heart failure. *Am J Physiol* 257:F515–F523, 1989
8. BURNETT JC JR, KAO PC, HU DC, HESER DW, HEUBLEIN D, GRANGER JP, OPGENORTH TJ, REEDER GS: Atrial natriuretic peptide elevation in congestive heart failure in the human. *Science* 231:1145–1147, 1986
9. CODY RJ, ATLAS SA, LARAGH JH, KUBO SH, COVIT AB, RYMAN KS, SHAKNOVICH A, PONDOLFINO K, CLARK M, CAMARGO MJF, SCARBOROUGH RM, LEWICKI JA: Atrial natriuretic factor in normal subjects and heart failure patients–Plasma levels and renal, hormonal, and hemodynamic responses to peptide infusion. *J Clin Invest* 78:1362–1374, 1986
10. ABASSI Z, POWELL J, GLOMB E, KEISER H: Renal and systemic effects of urodilatin in rats with high-output heart failure. *Am J Physiol* 262:F615–F621, 1992
11. ISNARD R, CARAYON A, EURIN J, MAISTRE G, BOUANANI N, BARTHELEMY C, CROZATIER B, KOMAJDA M, LEGRAND JC: Glomerular atrial natriuretic factor receptors in experimental congestive heart failure. *Am J Physiol* 265:H923–H928, 1993
12. SAITO Y, NAKAO K, ARAI H, SUGAWARA A, MORII N, YAMADA T, ITOH H, SHIONO S, MUKOYAMA M, OBATA K, YASUE H, OHKUBO H, NAKANISHI S, IMURA H: Atrial natriuretic polypeptide (ANP) in human ventricle. Increased gene expression of ANP in dilated cardiomyopathy. *Biochem Biophys Res Commun* 148:211–217, 1987
13. EISKJAER H, BAGGER JP, DANIELSEN H, JENSEN JD, JESPERSEN B, THOMSEN K, SORENSEN S, PEDERSEN EB: Mechanisms of sodium retention in heart failure: Relation to the renin-angiotensin-aldosterone system. *Am J Physiol* 260:F883–F889, 1991
14. VOLPE M, TRITTO C, DE LUCA N, MELE AF, LEMBO G, RUBATTU S, ROMANO M, DE CAMPORA P, ENEA I, RICCIARDELLI B, TRIMARCO B, CONDORELLI M: Failure of atrial natriuretic factor to increase with saline load in patients with dilated cardiomyopathy and mild heart failure. *J Clin Invest* 88:1481–1489, 1991
15. REDFIELD MM, EDWARDS BS, HEUBLEIN DM, BURNETT JC JR: Restoration of renal response to atrial natriuretic factor in experimental low-output heart failure. *Am J Physiol* 257:R917–R923, 1989
16. PETTERSSON A, HEDNER J, HEDNER T: Renal interaction between sympathetic activity and ANP in rats with chronic ischaemic heart failure. *Acta Physiol Scand* 135:487–492, 1989
17. GUTKOWSKA J, MUKADDAM-DAHER S, FUJIO N, KOCH C, TREMBLAY J: Characterization of lung ANF receptors in genetic cardiomyopathy. *Circulation* 88(Suppl):I315, 1993
18. TSUTAMOTO T, KANAMORI T, MORIGAMI N, SUGIMOTO Y, YAMAOKA O, KINOSHITA M: Possibility of downregulation of atrial natriuretic peptide receptor coupled to guanylate cyclase in peripheral vascular beds of patients with chronic severe heart failure. *Circulation* 87:70–75, 1993
19. SCHIFFRIN EL: Decreased density of binding sites for atrial natriuretic peptide on platelets of patients with severe congestive heart failure. *Clin Sci* 74:213–218, 1987
20. LEVIN ER, FRANK HJL, CHAUDHARI A, KIRSCHENBAUM MA, BANDT A, MILLS S: Decreased atrial natriuretic factor receptors and impaired cGMP generation in glomeruli from the cardiomyopathic hamster. *Biochem Biophys Res Commun* 159:807–814, 1989
21. YECHIELI H, KAHANA L, HARAMATI A, HOFFMAN A, WINAVER J: Regulation of renal glomerular and papillary ANP receptors in rats with experimental heart failure. *Am J Physiol* 265:F119–F125, 1993
22. CACHOFEIRO V, SCHIFFRIN E, CANTIN M, GARCIA R: Glomerular and vascular atrial natriuretic factor receptors in cardiomyopathic hamsters: Correlation with the peptide biologic effects. *Cardiovasc Res* 24:843–850, 1990
23. GARCIA R, BONHOMME MC, SCHIFFRIN EL: Divergent regulation of atrial natriuretic factor receptors in high-output heart failure. *Am J Physiol* 263:H1790–H1797, 1992
24. TREMBLAY J, FUJIO N, GOSSARD F, BAYARD F, GUTKOWSKA J, HAMET P: Determination of guanylate cyclase subtype expression by quantitative PCR in health and disease, in *Council for High Blood Pressure Research 47th Annual Fall Conference and Scientific Sessions*, Anonymous, San Francisco, 1993, (abstract)
25. CANAAN-KUHL S, JAMISON R, MYERS B, PRATT R: Identification of β-receptor for natriuretic peptide in human kidney. *Endocrinology* 130:550–552, 1992
26. KOLLER KJ, LOWE DG, BENNETT GL, MINAMINO N, KANGAWA K, MATSUO H, GOEDDEL DV: Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP). *Science* 252:120–123, 1991
27. MUKADDAM-DAHER S, GUTKOWSKA J, TREMBLAY J, DAM TV, QUILLEN EW: Regulation of renal atrial natriuretic factor receptors in the pregnant sheep. *Endocrinology* (in press)
28. JASMIN G, PROSCHEK L: Hereditary polymyopathy and cardiomyopathy in the Syrian hamster - 1. Progression of heart and skeletal muscle lesions in the UM-X7.1 line. *Circulation* 83:191–201, 1991
29. GUTKOWSKA J: Radioimmunoassay for atrial natriuretic factor. *Nucl Med Biol* 14:323–331, 1987
30. TREMBLAY J, HUOT C, WILLENBROCK RC, BAYARD F, GOSSARD F, FUJIO N, KOCH C, KUCHEL O, DEBINSKI W, HAMET P: Increased cyclic guanosine monophosphate production and overexpression of atrial natriuretic peptide A-receptor mRNA in spontaneously hypertensive rats. *J Clin Invest* 92:2499–2508, 1993
31. QUIRION R, DALPE M, DAM TV: Characterization and distribution of receptors for the atrial natriuretic peptides in mammalian brain. *Proc Natl Acad Sci USA* 83:174–178, 1986
32. CHOMCZYNSKI P, SACCHI N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
33. KOLLS J, DEININGER P, COHEN JC, LARSON J: cDNA equalization for reverse transcription-polymerase chain reaction quantitation. *Anal Biochem* 208:264–269, 1993
34. FUJIO N, GOSSARD F, BAYARD F, TREMBLAY J: Regulation of natriuretic peptide receptor A and B expression by transforming growth factor-β_1 in cultured aortic smooth muscle cells. *Hypertension* 23:908–913, 1994
35. NAKAO K, OGAWA Y, SUGA S, IMURA H: Molecular biology and biochemistry of the natriuretic peptide system II: Natriuretic peptide receptors. *J Hypertens* 10:1111–1114, 1992
36. TERADA Y, TOMITA K, NONOGUCHI H, YANG T, MARUMO F: PCR localization of C-type natriuretic peptide and B-type receptor mRNA in rat nephron segment. *Am J Physiol* 267:F215–F222, 1994
37. LATCHMAN DS: *Gene Regulation-A Eukaryotic Perspective*. London, Unwin Hyman Ltd., 1990, 269 pp
38. YAMAMOTO T, FENG L, MIZUNO T, HIROSE S, KAWASAKI K, YAOITA E, KIHARA I, WILSON C: Expression of mRNA for natriuretic peptide receptor subtypes in bovine kidney. *Am J Physiol* 267:F318–F324, 1994
39. REIS AM, FUJIO N, DAM T-V, MUKADDAM-DAHER S, JANKOWSKI M, TREMBLAY J, GUTKOWSKA J: Characterization and distribution of natriuretic peptide receptors in the rat uterus. *Endocrinology* (in press)
40. BROWN J, ZUO Z: Renal receptors for atrial and C-type natriuretic peptides in the rat. *Am J Physiol* 263:F89–F96, 1992
41. LUK JKH, WONG EFC, WONG NLM: Absence of C-type natriuretic peptide receptors in hamster glomeruli. *Nephron* 67:226–230, 1994
42. HAMET P, TREMBLAY J, PANG SC, SKUHERSKA R, SCHIFFRIN EL, GARCIA R, CANTIN M, GENEST J, PALMOUR R, ERVIN FR, MARTIN S, GOLDWATER R: Cyclic GMP as mediator and biological marker of atrial natriuretic factor. *J Hypertens* 4(Suppl 2):S49–S56, 1986
43. CHEVALIER RL, THORNHILL B, GOMEZ RA, RAGSDALE NV, PEACH MJ, CAREY RM: Role of atrial natriuretic peptide in the response to blood volume expansion in the weanling rat. *Pediatr Res* 27:396–400, 1990
44. DE LEON H, GAUQUELIN G, GARCIA R: Angiotensin II directly down regulates glomerular ANF receptors. *Hypertens Res* 16:57–63, 1993
45. TSUNODAH K, MENDELSOHN FAO, SEXTON PM, CHAI SY, HODSMAN

GP, JOHNSTON CI: Decreased atrial natriuretic peptide binding in renal medulla in rats with chronic heart failure. *Circ Res* 62:155–161, 1988

46. VALENTIN JP, QUI C, MULDOWNEY W, YING W-Z, GARDNER D, HUMPHREYS M: Cellular basis for blunted volume expansion natriuresis in experimental nephrotic syndrome. *J Clin Invest* 90:1302–1312, 1992
47. MUKADDAM-DAHER S, GUTKOWSKA J, NUWAYHID BS, QUILLEN EW JR: Metabolic clearance of atrial natriuretic factor in ovine pregnancy. *Am J Physiol* 267:R1413–R1420, 1994
48. BERG JA, HAYASHI M, FUJII Y, KATZ AI: Renal metabolism of atrial natriuretic peptide in the rat. *Am J Physiol* 255:F466–F473, 1988
49. RITTER D, NEEDLEMAN P, GREENWALD JE: Synthesis and secretion of an atriopeptin-like protein in rat cell culture. *J Clin Invest* 87:208–212, 1991
50. VALENTIN JP, SECHI LA, QIU C, SCHAMBELAN M, HUMPHREYS MH: Urodilatin binds to and activates renal receptors for atrial natriuretic peptide. *Hypertension* 21:432–438, 1993
51. GAGELMANN M, HOCK D, FORSSMANN WG: Urodilatin (CDD/ANP-95–126) is not biologically inactivated by a peptidase from dog kidney cortex membranes in contrast to atrial natriuretic peptide/cardiodilatin (-hANP-99–126). *FEBS Lett* 233:249–254, 1988
52. LEGAULT L, CERNACEK P, LEVY M, MAHER E, FARBER D: Renal tubular responsiveness to atrial natriuretic peptide in sodium-retaining chronic caval dogs. *J Clin Invest* 90:1425–1435, 1992
53. SHOWALTER CJ, ZIMMERMAN RS, SCHWAB TR, EDWARDS BS, OPGENORTH TJ, BURNETT JC: Renal response to atrial natriuretic factor is modulated by intrarenal angiotensin II. *Am J Physiol* 254: R453–R456, 1988
54. SMITH BJ, LINCOLN TM: Angiotensin decreases cyclic GMP accumulation produced by atrial natriuretic factor. *Am J Physiol* 253:C147–C150, 1987